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Evaluation of the antimicrobial activity of Moringa oleifera leaves extract on Helicobacter pylori

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Abstract: *The present study was carried out to determine the antimicrobial activity of aqueous and methanol extracts of Moringa oleifera leaves on H. pylori. Disc diffusion method as described by Kirby-Bauer was used to determine the antimicrobial activity of the extracts which were compared with that of the standard antibiotics. The susceptibility profiles of H. pylori isolates were determined using standard antibiotics, aqueous and methanol extracts of Moringa oleifera leaves. Out of 487 positive isolates (75.73%), 350 (71.87%) were sensitive to Clarithromycin with 30mm mean zone of inhibition, 304 (62.42%) were sensitive to Tetracycline (27mm), 266 (54.62%) to Amoxicillin (28mm), 173 (35.52%) to Metronidazole (25mm), while all isolates were resistant to Ciprofloxacin. The Minimum Inhibitory Concentration (MIC) of aqueous Moringa oleifera leave extracts ranged from 0.035µg/ml to 0.05µg/ml with MBC of 0.0425µg/ml while the MIC of methanol extract ranged from 0.0425µg/ml to 0.05µg/ml with MBC of 0.0425. These results clearly suggest that Moringa oleifera leaves act as potent growth inhibitor of H. pylori.*

Keywords: *Antimicrobial activity, Moringa oleifera, Helicobacter pylori*

I. Introduction

The use of extracts from medicinal plants as antimicrobial agents has been in existence over the years. The antimicrobial properties of plants have been investigated by a number of studies as many of them have been used as therapeutic alternatives due to their antimicrobial properties (Adriana et al., 2007). Plant extracts contain not only minerals and primary metabolites but also a diverse array of secondary metabolites with antioxidant potentials (Sofowara, 1993) which have shown antimicrobial properties. Moringa oleifera is a highly valued plant, distributed in many countries in the tropics and sub-tropics. It has an impressive range of medicinal uses with high nutritional value (Abalaka et al., 2012). Thus, it is necessary to determine the antimicrobial properties and the phytochemical composition of Moringa oleifera. This plant is referred to a number of names such as miracle tree, drumstick tree and horse radish tree.

Helicobacter pylori plays a major role in the development of gastritis, peptic ulcer, gastric mucosa associated lymphoid tissue (MALT) lymphoma and gastric cancer (Borislav, 2011). There has been an increase in drug resistance and treatment failure against H. pylori and researchers are trying to find medicinal plants that could be used as alternative source of antimicrobials which has low potential to cause resistance Alam et al., 2009).

II. Materials And Method

Collection and preparation of plant materials

Fresh leaves of Moringa oleifera were collected from Moringa tree located at Orba, in Udenu Local Government Area of Enugu State, Nigeria. They were washed using sterile distilled water and air-dried at room temperature. The dried leaves were ground to fine powder using electric blender. 100mg, 85mg, 70mg, 55mg and 40mg of Moringa oleifera leaf powder were soaked in 100 ml of distilled water and methanol separately. The aqueous mixtures were kept for 24 hours with constant agitation at 30 minutes intervals, while the methanolic mixtures were left at room temperature for 48 hours also with constant agitation. These were filtered using sterile Whatman No.1 filter paper. The extracts obtained were stored in a refrigerator at 4^{o}C for further use (Bukar et al., 2010).

Preparation of the disc for antimicrobial sensitivity test using the extracts

Discs of about 6.35mm diameter were made from Whatman's No.1 filter paper using a paper puncher. The discs were transferred into Bijou bottles and sterilized by autoclaving at 121°C for 15minutes. The sterilized discs were soaked in different concentrations of the extracts 0.1µg, 0.085µg, 0.07µg, 0.055µg and 0.04µg (aqueous and methanol extracts of Moringa oleifera) (Bukar et al., 2010).

Preparation of media

Thirty eight (38) g of Mueller Hinton dehydrated media (Merck Co Germany) was suspended in 1000 ml of distilled water. It was dispensed in 500ml conical flask and it was heated with frequent agitation and was

boiled for one minute, before sterilizing at 121° C for 15 minutes. It was allowed to cool to 45-50°C and 25 ml of defibrinated sheep blood was added before dispensing into sterile Petri dishes.

Antimicrobial Analysis

Disc diffusion method as described by Kirby-Bauer (2009) was used to determine the antimicrobial activity of the extracts.

Plates containing Mueller-Hinton agar with defibrinated sheep blood were inoculated with 10^7 CFU of H. pylori, which was evenly swabbed on the entire surface of each of the plates respectively. The turbidity of the inocula was adjusted using 0.5 McFarland turbidity Standard. Using an ethanol dipped and flamed forceps, the discs containing the various concentrations of the Moringa oleifera leave extracts were aseptically placed individually over the seeded agar plates. They were separated from each other to avoid overlapping of inhibition zones. Amoxicillin (Abtek, UK) was used as the positive control. The discs were gently pressed down onto the agar and the agar plates were inverted and incubated microaerophilically at 37°C for 2 – 5 days. Clear zones of inhibition produced by the organisms were observed and measured. The zones of inhibition of the various extracts were compared to that of the standard drug. The test was done in triplicate and the mean diameter of zones of inhibition was determined.

Sensitivity test using antibiotics

Susceptibility testing was performed to commonly used antibiotics including Clarithromycin 10µg, Tetracycline 25µg, Amoxicillin 25µg, Metronidazole 30µg and Ciprofloxacin 10µg (Abtek, UK) as described by National Committee for Clinical Laboratory Standards (NCCLS) (1993). E. coli (NCTC. 10418) was used as a control organism.

Determination of Minimum Inhibitory Concentration (MIC)

This was done using Moringa oleifera leave extract. Two fold serial dilutions of the extract were prepared in test tubes containing Mueller-Hinton broth as diluent according to NCCLS (1993) guidelines. Each dilution was seeded with the organism to the standard concentration of 10^7cfu/ml, and incubated microaerophilically at 37°C for 2 - 3 days. Test tubes containing Mueller Hinton broth only and Mueller Hinton broth with the extract were used as control. They were also incubated microaerophilically at 37°C for 2 - 3 days. The Minimum Inhibitory Concentration was taken as the lowest concentration of the extract that shows no visible growth or completely inhibits the growth of the organism, after microaerophilic incubation at 37°C for 2 - 3 days (Abalaka et al., 2012).

NB: Preparation of Mueller Hinton Broth

Twenty one (21) g of Mueller Hinton broth was suspended in 1000 ml of distilled water. It was dispensed in 500ml conical flask and was sterilized at 121° C for 15 minutes. It was allowed to cool before it was used for the serial dilution.

Determination of Minimum Bactericidal Concentration (MBC)

It was determined by aspirating 0.1ml of the culture medium from each of the test tubes showing no apparent growth or visible turbidity after microaerophilic incubation at 37°C for 2 - 3 days. It was sub cultured onto fresh Mueller-Hinton Agar plates with 5% defibrinated sheep blood and re-incubated microaerophilically at 37°C for 2 – 5 days. The MBC was determined as the lowest concentration of the extract that shows no visible growth after the incubation (Abalaka et al., 2012).

III. Results

Table 1: Antimicrobial susceptibility pattern of Helicobacter pylori using antibiotics

Antimicrobial agent	Number of samples	Number sensitive	Number resistance	Disc potency (µg)	Zone of inhibition (mean) (mm)
Clarithromycin	487	350	137	10	30
Amoxycillin	487	266	221	25	28
Metronidazole	487	173	314	30	25
Tetracycline	487	304	183	25	27
Ciprofloxacin	487	0	487	10	0

Table 2: Antimicrobial activity of different concentrations of Moringa oleifera leaf aqueous extract on Helicobacter pylori

Concentration (µg /ml)	Zone of inhibition (mm)
0.1	32
0.085	30
0.07	24
0.055	18
0.04	11

Table 3: Antimicrobial activity of different concentrations of Moringa oleifera leaf methanol extract on Helicobacter pylori

Concentration (µg /ml)	Zone of inhibition (mm)
0.1	28
0.085	23
0.07	18
0.055	10
0.04	7

Table 4: Minimum Inhibitory Concentration of Moringa oleifera aqueous leave extract on Helicobacter pylori

Concentration (µg /ml)	0.05	0.0425	0.035	0.0275	0.02
MIC	+	+	+	-	-

NOTE: + Indicates Inhibition of the organism
- Indicates growth of the organism

Table 5: Minimum Inhibitory Concentration of Moringa oleifera methanol leave extract on Helicobacter pylori

Concentration (µg /ml)	0.05	0.0425	0.035	0.0275	0.02
MIC	+	+	-	-	-

NOTE: + Indicates Inhibition of the organism
- Indicates growth of the organism

IV. Discussion

During the studies, it was observed that 487 samples were tested for antimicrobial sensitivity and 350 samples were sensitive to Clarithromycin, 266 were sensitive to Amoxycillin, 173 samples were sensitive to Metronidazole and 304 were also sensitive to Tetracycline. All the clinical isolates were resistant to Ciprofloxacin (table 1).

Antimicrobial sensitivity tests were also carried out using different concentrations of aqueous and methanol extracts of Moringa oleifera leaves. It was observed that the aqueous extracts exhibited more antibacterial activity than the methanol extracts (tables 2 & 3).

The MIC of aqueous Moringa oleifera leaf extracts ranged from 0.035µg/ml to 0.05µg/ml with MBC of 0.0425µg/ml while the MIC of methanol extract ranged from 0.0425µg/ml to 0.05µg/ml with MBC of 0.0425. These results clearly confirm that Moringa oleifera leaves act as potent growth inhibitor of H. pylori (tables 4 & 5).

Treatment / eradication of H. pylori using antibiotic therapy are presently used in the management of the infection. Due to treatment failure, contraindications, side effect, drug resistance and high cost of drugs, Moringa oleifera leaves have been found to be effective alternative therapy against H. pylori infections.

V. Conclusion

It was observed that there was high antibiotic resistance during the research. This could be due to the use of the drug in the treatment of gynaecologic, dental and gastrointestinal infections. Hence, the occurrence of this organism can be controlled using the leaves of Moringa oleifera as alternative therapeutic agent against H. pylori infections instead of antibiotics.

References

[1]. Abalaka, M. E., Daniyan , S. Y., Oyeleke, S.B., and Adeyemo, S.O. (2012). The Antibacterial Evaluation of Moringa Oleifera Leaf Extracts on Selected Bacterial Pathogens. Journal of Microbiology Research, 2(2): 1-4.
[2]. Adriana, B., Almodovarl, A.N.M., Pereiral, C.T. and Mariangela, T.A. (2007). Antimicrobial Efficacy of Curcuma zedoaria extract as Assessed by Linear Regression Compared with Commercial Mouthrinse. Braz. J.Microbiol. 38: 440-445.
[3]. Alam, M.F., Mashiar, M., Rahman, M., Mominul, I.S., Shamima, A.S., Soriful, M.I., Rahman, M.A., and Mizanur, R.M. (2009) Antibacterial Activity of Leaf Juice and Extracts of Moringa oleifera Lam. Against Some Human Pathogenic Bacteria CMU. J. Nat. Sci. Vol. 8 (2).
[4]. Borislav, V. (2011). Helicobacter pylori. Horizon Scientific Press. P. 289
[5]. Bukar, A., Uba, A. and Oyeyi, T. I. (2010). Antimicrobal Profile of Moringa oleifera Lam. Extracts against Microorganisms. Journal of Microbiology, 8(2): 59-67.
[6]. Kibry, W. M. M., Bauer, A. W., Sherris, J.C. and Turck, M. (2009). Antibiotic Susceptibility Testing by a Standardized Single Disc Method. American Journal of Clinical Pathology, 45: 493-496.
[7]. National Committee for Clinical Laboratory Standards (1993). Methods for Dilution of Antimicrobial Susceptibility Tests for Bacteria. NCCLS Villanov.
[8]. Sofowora, A. (1993). Medicinal Plants and Medicine in Africa. Spectrum Books, Ibadan, Nigeria. p. 120-123.